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Dear Josh,

The reprints finally arrived this morning. I was all set to call W &W for after receipt of your letter yesterday I feared we were ti have even greater difficulties. I have received to date some fifty requests which with your 22 and some 20 to be distributed here will leave about 100. Unfortunately our departmental secretary is on a two eek vacation and your plan can not be put into operation. I could send you all of the cards and the seventy-five reprints to cover them plus the 25 or so that you need or I colud just send you the cards have you check them and return them to me for distribution. I shall await your reply before doing anything further with them other than local distribution.

The new manuscript which you propose has been on my mind for some time now. It should be written both because of vagueries in the first and the fact that the hypothesis has now reached a simplified state that is worthy of explicit statement. I've enclosed a tentative outline for the paper. It is in reality nothing more than the enumeration of the various points we can bring to bear. I shall as soon as possible start writking up the varixous sections that are already completeand getting the data in useful tables. I too have the impression that the general consistency of the story has caused me at times to gloss over certain aspects and move on t o another before securely settling any particular point. Some bactracking may be necessary but it would be wiser to do it now before my notes become totally uncomprehendable. The material * is in fairly good shape while the ! is either incomplete or not yet done. You may add or subtract any particular section.

As to further work. -- I should be gald to get the thermal inactivation rate--- on differential centrifugation the only data available are those obtained during the course of purification of plate lysates with alternating cycles of high and low speed centrifugation and the recovery of FA remaining in constant ratio to the phage. --- the rate of phage and FA adsorption has not been done as both can not be measured in a single experiment. With the AS we could stop the adorption at any particular time but only phage or FA recovery could be measured in any particular system. Thus the lytic variant could be used for phage and the parent phage for FA on a phage sensitive assay system. Probably one of the most informative ways of * settling the cohort problem is an experiment a là Hershey. I shall be out to CSH some time in the near future and shall discuss this with him. -- For the U.V adsorption of the phage I should be able to get a complete curve as they have a recording spectrophotometer here.

While I think of it-- Since in some respects an MS is written for a journal how about J. Exp Med as I should be able to swing it from here and so ease editorial matters etc.

* and elegant

I couldn't help but be amused by the enclosure with your reprint. Edwards et al must be pulling their hair out by now. Hope Spicer can do the same for the somatic antigens. Have you kept a record of the phage to FA ratio of your various preps on LA-22 as I think this is worthy of inclusion. If you haven't I would be gald to carry this along. I have a few data on this point thus PLT-22 = PLT-22/2 =PLT-22/7 > PLT-22/547 and is < PLT-22/558 PLT-22/Ed 57. The extremes of the ratio grows on different strains or affcecting different characters has been 10^{0} to 10^{7}, certainly nowhere near the values I'd like.

My U.V. inactivation data are as follows; the phage is inactivated 45 %/minute through three decades and then the curve breaks sharply downward, the first part seems to have a soft slope rather than be linear; the FA as assayed on LA-22 Aux to Prot has a slope of 18 %/min. after the activation and was followed only through one decade.(9 minutes). Your data are therefore not too discrepant with mine, as some changes might have occurred in the output of the lamp or different conditions of irradiation.

As you probably by now realize your 22V and mine are comparable. Sorry to have been so obfuse and obtuse. The mutant is quite frequent and is about one percent of the phage secreted by LT-22. It is not readily seen on LT-2 unless plating conditions are just right but scores beautifully on S. gal (Didn't I send you some pictures of it?). It seems to be a typically lytic phage although I've not yet tested whether U.V. inactivated it still kills. Am now running its inactivation curve for comparison with the parent. On LA-22 I also observed what I thought to be a low transducing ability and in fact was concerned as to whether it or some contaminating reverse mutations were responsible for the transductions. It seems to adsorb as well as the parental reaching the same saturation level of about 8/ bacterium but unlike the parental where the number of transductions is independent of the multiplicity here they are dependent. That is at dilution 0.1 per to 1 per the line is linear and the phage FA ratio is $10^{7}/1$,above one the curve starts braeking and bends around with actually a lower yield at five than one. As I mentioned thirty tested transinductions all secreted the parental phage not the mutant. If we assume that the replacement of the parental phage by the mutant results in the death of the cell a la Bertani the results are straightforward. However I have not been able to show explicitly the death of any portion of the population so infected. Then again such death may not result in the loss of a clone and by taking into consideration the fact that, as you mention, transduced clones are ordinarily * pure lysogenic and that there is probably a delay in the clonalization of a transduction only transduced cells where there has been phage replacement can die. This results in losses of transductions but not of infected clones. I hope I've made this reasoning clear for it is the same line of reasoning one can use to explain the U.V. activation. You may remember we calculated the predicted peak on the basis that the probability of a transduction was composed of the probability of the adsorption of the proper particle as a function of dose and the probability that the phage did not kill as a function of dose. The results fit the observed peak but necessitated that some 90 % of the cells die which we could not demonstrate to occur. However 90% of transduced cells could die with no clonal losses demonstrable.

* phage + FA go together unless until forced to separate by either
a) prophage exclusion 22V
b) [illegible] low efficiency of [illegible] 666

Transduction by lytic variants should also be tested on a synthetis assay system (a sensitive made lysogenic for the parental phage) as here the role of multiplicity should be even clearer due to the larger numbers of particles that can be adsorbed. Also to be tested is the role of U.V in such a system. One experiment with U.V'd 22V , multiplicity slightly less than one, indicates U.V inactivated phage kills sensitive cells, however a simultaneous experiemnet with 22 also killed (Used a dose adequate for 22 , am now running killing curve of 22 V). If you've not yet run your protection experiemnt might I suggest you use SW-188 as it is far stabler than any LT-2 derivative I had.

Let me wish you and all in the lab a happy New Year.

Sincerely,

Norton